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EXAMINER	
WALICKA, MALGORZATA A	
ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 05/27/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/308,207	DUNN-COLEMAN ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *Dec.15, 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *19-21,26-31 and 41-51* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *19-21,26-31 and 41-51* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *12/15/03*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

The Amendment and Response to Office Action filed on Dec. 15, 2003 is acknowledged. The amendments to the claims have been entered as requested. Claims 1-18 are previously cancelled. Claims 22-25 and 32-40 are currently canceled. Claims 19-21 and 28 are amended. Claims 41-51 are new.

Claims 19-21, 26-31 and 41-51 are pending and are the subject of this Office Action.

Detailed Office Action

1. Objections

The title of Example 1 is objected to because it contains the phrase "expression of 1,3-propanediol". 1,3-propanediol is a chemical that cannot be expressed.

The address of ATCC on page 30 is not corrected.

The specification is objected to because description of Figure 2A-2G, page 8, line 29, is confusing. Protein X encoded by gene dhab4 as presented in Figure 2 is not that of SEQ ID NO: 59. Although at least 20 N-terminal and at least 20 C-terminal amino acids of both sequences are identical, the protein set forth by SEQ ID NO: 59 consist of 607 amino acids, whereas the protein DHAB4 whose amino acid sequence is presented Figure 2 consists of 727 amino acids.

ATCC numbers 69789 and 69790 quoted on page 9 line30 and 33 are not valid. Please provide the correct ATCC numbers.

2. Rejections

2.1. 35 USC 112, second paragraph

Rejection of claim 21, made in the previous Office Action, Oct. 6, 2003, as confusing in recitation of the phrase "The recombinant microorganism of Claim 19 selected from the group consisting of *Citrobacter, Enterobacter, etc.*" is withdrawn, because the claim has been amended.

Rejection of claims 29-31 for recitation of the limitations "protein 1", "protein 2" and "protein 3" is withdrawn, because the base claim 20 has been amended.

Rejection of claims 19-30 as confusing is now withdrawn, because claim 19 has been amended.

Claim 28 and 49 are rejected, because the phrase "as shown between positions 0749 –11572 of SEQ ID NO: 19" is confusing. It is unknown what residue in SEQ ID NO: 19 is numbered 0749; it should be 9749-11572 (page 11 of the specification).

Claim 45 is indefinite because it recites SEQ ID NO: 66 as an amino acid sequence. SEQ ID NO: 66 is a nucleotide sequence.

Claim 19, 20-21, 26-31, 41-51 are rejected because it is unclear whether production of 1,3 -propanediol in the recombinant microorganism is greater than in the not transformed counterpart only due to the introduction of the gene encoding X-protein or also because a glycerol–3-phosphatase and glycerol or diol dehydratase were introduced, or because the gene encoding X-protein and glycerol or diol dehydratase were introduced.

2.2. 35 U.S.C. section 112, first paragraph paragraph

2.2.1. Lack of written description

Claims 19-21, 26-31 and 41-51 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 19-21, 26-31 and 41-51 are generic, because they are directed to a large and variable genus of recombinant microorganisms transformed with the following subgenera of genes:

a) any gene encoding a glycerol or diol dehydratase activity,

b) any gene encoding a glycerol–3 phosphatase, and

c) gene encoding a "protein X" wherein the gene is isolated form a glycerol dehydratase gene cluster of *Klebsiella* and *Citrobacter*, or isolated form a diol dehydratase gene cluster from *Klebsiella, Clostridium* and *Salmonella*.

The claims are directed to large and versatile genus of transformants transformed with large and versatile genera of glycerol or, diol dehydratases and with a genus of glycerol–3-phosphatase. Providing glycerol dehydratase from *Klebsiella* and *Citrobacter* does not provide an identifying characteristics of all glycerol dehydratases. Similarly, providing diol dehydratase form Klebsiella, Clostridium, and Salmonella, does not provide the identifying characteristics the whole genus of diol dehydratases. The disclosure misses description of structure or other identifying characteristics of other

glycerol or diol dehydratases genes used for transformation. Also, the two representative species of glycerol–3-phosphatases (SEQ ID NO: 17 and 33) do not possess a characteristics identifying any glycerol–3-phosphatase.

The disclosure misses description of structure, or other identifying characteristics, of genes encoding protein X of glycerol dehydratase regulon for all the species of genus *Klebsiella* and Citrobacter. The specification does not teach diol dehydratase genes for all the species of genus *Klebsiella*, *Citrobacter* and *Salmonella*. It is not clear how one skilled in the art can recognize a protein X gene in a glycerol or diol dehydratase cluster of all the organisms in recited genera. Providing one representative species of protein X gene from *Klebsiella pneumoniae*, *Citrobacter freundii* and *Salmonella tiphimurium* does not allow for identification of all these genes from genus *Klebsiella, Citrobacter* and *Salmonella*. The structure of recited genes encoding protein X is much clearer described by claim 49 than by claim 19.

Furthermore, it is not clear which of extremely large number of diol or glycerol dehydratases and glycerol-3 phosphatases produced by any living organism or man-made are included or excluded from the scope of invention. The specification does not explain if any glycerol and diol dehydratase needs reactivation for its full activity, and if so, whether the X protein and protein 1, 2, 3, from glycerol dehydratase regulon of Klebsiella pneumoniae and protein X from diol dehydratase gene cluster from Klebsiella, Clostridium, and Salmonella are able to reactivate any glycerol/diol dehydratase. Similarily it is unknown if any glycerol or diol dehydratase from any

organism requires a reactivation process in which proteins 1, 2, 3 from glycerol dehydratase regulon of Klebsiella pneumonie act.

Claims 47-51 are generic and directed to *E. coli* transformed with glycerol or diol dehydratase, however, the specification provides only representative species of the genes encoding glycerol dehydratase form *Klebsiella* and *Citrobacter* and representative species of the diol dehydratase gene from *Klebsiella, Citrobacter* and Salmonella. Theses genes are not representative for the versatile genera of all glycerol and diol dehydratases. The claims and disclosure are missing description of structure of genes that were used for transformation or other identifying characteristics of such genes. It is not clear which one of extremely large number of glycerol or diol dehydratases produced by any living organism or man-made are included or excluded from the scope of invention.

In addition, claims 20, 29-31, 48, and 50 are rejected because these claims recite genera of genes encoding "protein 1", "protein 2" and "protein 3", which are diverse in functional characteristics. The claims do not recite the function of proteins of SEQ ID NO: 60, 61, 62, 63, 64 and 65 and proteins that are at least 95% similar to these sequences. Applicants fail to disclose any particular structure to function/activity relationship for polynucleotide encoding amino acid SEQ ID NOs: 60, 61, 62, 63, 64 and 65, and the specification fails to teach how to modify initial nucleotide sequences so that the proteins having 95% similarity retained the functions of protein of SEQ ID NOs: 60, 61, 62, 63, 64 and 65.

Given the lack of functional characteristics of representative species as encompassed by the claims, Applicants have failed to sufficiently describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize Applicants were in possession of the claimed invention when the application was filed.

Furthermore, claim 28 is reciting gene encoding protein X as the one consisting of nucleotides 0749-11572 of SEQ ID NO: 19. Assuming that "0" in 0749 is a typing error, as "9" and "0" are next to each other on the keyboard, i.e. the proper number is 9749, one can calculate that the gene consist of 1724 nucleotides. The longest polypeptide encoded by 1724 nucleotides consists of 574 amino acids. This is not 727 amino acids as in case of protein from FIG. 2A-2G, nor 607 amino acids as in case of SEQ ID NO: 59. In addition, although nucleotides having number 11572 and less encode the C-terminus of SEQ ID NO: 59 or DHAB4 protein of FIG. 2, nucleotides 9749 and further do not encode N-terminus of SEQ ID NO: 59 or DHAB4 protein of FIG. 2. As such, the actual sequences of the gene encoding protein X of *Klebsiella pneumonie* and protein X itself are unknown.

Claim 45 is directed to a microorganism transformed with the gene encoding protein X of SEQ ID NO: 59. Protein X of SEQ ID NO: 59 is, according to the description to FIG 2A-2G on page 8 line 29, encoded by gene dhab4. However, although at least 20 N-terminal and at least 20 C-terminal amino acids of both sequences are identical, the protein set forth by SEQ ID NO: 59 consist of 607 amino acids, whereas the protein DHAB4 whose amino acid sequence is presented Figure 2

consists of 727 amino acids. As such, the actual sequences of the gene encoding protein X and protein X itself are unknown.

In conclusion, the gene encoding protein X from the glycerol dehydratase of *Klebsiella pneumoniae* used by inventors to make the claimed transformants is not described in such full, clear, concise, and exact terms as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 19-21, 26-31 and 41-46 are directed to a transformed microorganism comprising:

a) at least one introduced gene encoding a glycerol or diol dehydratase activity,

b) at least one introduced gene encoding a **glycerol-3-phosphatase**; and

c) at least one gene encoding protein X.

Upon the reconsideration of the disclosure, the examiner concludes that the claimed transformants were not in possession of the inventors when the application was filed.

None of the transformants presented in Examples 1, 2, 3, 5, 6 and 10 comprises the introduced set of a)-c) genes. Transformants of example 10 are *E. coli* cells containing plasmid pHK 28-26. According to description of plasmid pHK 28-26, page 31, line 21 and further is as follows:

"A 12.1 kb EcoRI-SalI fragment from pKP1, subcloned into pIB31 (IBI Biosystem, New Haven, CT), was sequenced and termed pHK28-26 (SEQ ID NO: 19). Sequencing revealed the loci of the relevant open reading frames of the dha operon encoding glycerol dehydratase and genes necessary for regulation. Referring to SEQ ID NO: 19, a fragment of the open reading frame for dhaK encoding dihydroacetone kinase is found at bases 1-399; the open reading frame dhaD encoding glycerol dehydrogenase is found at bases 983-2107; the open reading frame dhaR encoding the repressor is found at bases 2209-4134; the open reading frame dhaT encoding 1,3-propanediol oxidoreductase is found at bases 5017-6180; the open reading frame dhaB1 encoding the alpha subunit glycerol dehydatase is found at bases 7044-8711; the open reading frame dhaB2 encoding the beta subunit glycerol dehydratase is found at bases 78724-9308; the open reading frame dhaB3 encoding the gamma subunit glycerol dehydratase is found at bases 9311-9736; and the open reading frame dhaBX, encoding a protein of unknown function is found at bases 9749-11572."

According to the quoted description, plasmid pHK28-26 does contain a DNA encoding *Klebsiella* dehydratase dhaB 1, 2, 3 and reactivating factor X, but does not contain any glycerol-3-phosphatase.

In conclusion, one skilled in the relevant art is not convinced that Applicants, at the time the application was filed, had possession of the claimed invention.

2.2.2. Scope of enablement

Rejection of claim 19-31 under 35 U.S.C. 112, first paragraph made in the previous Office Action, Oct. 6, 2003, is withdrawn, because the amended claims do not recite 50% similarity to protein X and claims 22-25 are canceled.

Claim 20–22, 47, 50 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for microorganisms transformed with DNA molecule encoding polypeptides SEQ ID NO: 60, 61, 62, 63 64 and 65, does not reasonably provide enablement for microorganism transformed with DNA molecules encoding proteins that are in at least 95% similar to polypeptides of SEQ ID NO: 60, 61, 62, 63, 64 and 65, because the function of these polypeptides is unknown. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are broader than the enablement provided by the disclosure with regard to extremely large number of microorganisms transformed with polynucleotides encoding polypeptides having at least 95% similarity to polypeptides of SEQ ID NO: 60, 61, 62, 63 64 and 65; see the above rejection for lack of written description.

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention. Factors to be considered in determining whether undue experimentation is required, are summarized *In re* Wands

[858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)]. The Wands factors are: (a) the nature of the invention, (b) the breadth of the claim, (c) the state of the prior art, (d) the relative skill of those in the art, (e) the predictability of the art, (f) the presence or absence of working example, (g) the amount of direction or guidance presented, (h) the quantity of experimentation necessary.

The nature and breath of the claimed invention encompasses any microorganism that is transformed with at least one DNA encoding:

a) a protein having at least 95% similarity to the protein of SEQ ID NO: 60,

b) a protein having at least 95% similarity to the protein of SEQ ID NO: 61,

c) a protein having at least 95% similarity to the protein of SEQ ID NO: 62,

d) a protein having at least 95% similarity to the protein of SEQ ID NO: 63,

e) a protein having at least 95% similarity to the protein of SEQ ID NO: 64, and

f) a protein having at least 95% similarity to the protein of SEQ ID NO: 65.

While methods of gene cloning and gene structure manipulations are well known in the relevant art, skills of the artisans highly developed, the lack of the functional and structural characteristics of said polynucleotides makes the probability of success in obtaining the claimed invention very low. The claims necessitate selecting, from all natural or man made sources, DNA molecules that supposedly encode

a) a protein having at least 95% similarity to the protein of SEQ ID NO: 60,

b) a protein having at least 95% similarity to the protein of SEQ ID NO: 61,

c) a protein having at least 95% similarity to the protein of SEQ ID NO: 62,

d) a protein having at least 95% similarity to the protein of SEQ ID NO: 63,

e) a protein having at least 95% similarity to the protein of SEQ ID NO: 64, and

f) a protein having at least 95% similarity to the protein of SEQ ID NO: 65,

sequencing them, selecting those that do have properties a)-f), expressing them in an appropriate host and selecting those that act as proteins of SEQ ID NO: 60, 61, 62, 63, 64 or 65. While enablement is not precluded by a tedious experimentation, such experimentation has low probability of success absent teachings regarding the structure and function of the DNA molecules used for transformation or guidance how to modify the initial DNA molecules so that after modification they retained the desired property of encoding proteins having the same functions of the same as those of amino acid sequence of SEQ ID Nos: 60, 61, 62, 63, 64, 65 and 95% similarity to the original amino acid sequence.

Examiner concludes that without the further guidance on the part of Applicants regarding function and structure of the polynucleotides used for transformation, experimentation left to those in the art is improperly extensive and undue.

Claims 19-21, 26-31 and 41-51 are rejected for scope of enablement because the specification, while being enabling for a microorganism transformed with the set of genes

a) a gene encoding a glycerol dehydratase from *Klebsiella pneumonie* or *Citrobacter freundi* or diol dehydratase activity from *Klebsiella pneumonie*, *Clostridium* or *Salmonella typhimurium,*

b) a gene encoding a **glycerol-3-phosphatase** wherein said gene (genes GPP1 and GPP2) is isolated from ***Saccharomyces cerevisiae***; and

c) a gene encoding protein X from glycerol dehydratase gene cluster of *Klebsiella pneumonie* and *Citrobacter freundi* or diol dehydratase gene cluster of *Klebsiella pneumonie*, *Clostridium* or *Salmonella typhimurium,*

wherein production of 1,3-propanediol is greater in the recombinant microorganism comprising protein X than in the recombinant microorganism without protein X, and said production isfurther increased by introduction of protein1, 2, and 3, is not enabled for any glycerol dehydratase, any diol dehydratase and any glycerol phosphatase.

While enablement is not precluded by the necessity for routine screening, if a large amount of screening for the suitable enzyme is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed so that the claimed transformants functioned as intended by Applicants. In the instant case probability of obtaining the claimed inventions is low in the absence of teachings whether any glycerol dehydratase, for example a bovine glycerol dehydratase, or bovine diol dehydratase, needs to be reactivated for gaining their full activity and it so, whether protein X from glycerol dehydratase gene cluster of *Klebsiella pneumonie* and *Citrobacter freundi* or diol dehydratase gene cluster of from *Klebsiella pneumonie*, *Clostridium* and *Salmonella typhimurium,* and furthermore whether protein 1, 2, and 3, having structure of SEQ ID NO: 60, 61, 62, 63, 64, 65 or 95% similarity to these sequences will further increase

activity of the bovine glycerol dehydratase or bovine diol dehydratase in any type of microorganism.

Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. Without further guidance on the part of Applicants regarding glycerol and diol hydratases suitable for making the claimed invention those skilled in the art are forced to improper, extensive and undue experimentation.

3. Response to Applicants' traverse

Traversing the rejection under 35 USC section 112, first paragraph, Applicants write:

> "Subgenus (a) of claim 19 is now directed to genes encoding glycerol dehydratases and diol dehydratases. Specific glycerol dehydratases and diol dehydratases are taught in the specification...(see for example pages 2 and 10 of the disclosure). Subgenus b) is directed to the enzyme G3P and this enzyme is taught has being encoded [as being encoded?] by GPP1 and GPP2 genes."

Furthermore Applicants state,

> "With respect to subgenus c, amended claim 19 is directed to protein X isolated from glycerol dehydratase gene cluster from an organism selected from the genera

consisting of Klebsiella and Citrobacter or ii) isolated from a diol dehydratase gene cluster from an organism selected form the genera consisting of Klebsiella, Clostridium and Salmonella.

Applicants arguments have been fully considered but are found not persuasive, because the specification does not explain if any glycerol and diol dehydratase needs reactivation for its full activity, and if so, whether the X protein and protein 1, 2, 3, from glycerol dehydratase regulon of Klebsiella pneumoniae and protein X from diol dehydratase gene cluster from Klebsiella, Clostridium, and Salmonella are able to reactivate any glycerol/diol dehydratase.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka, Ph.D., whose telephone number is (703) 305-7270. The examiner can normally be reached Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, Ph.D. can be reached on (703) 308-3804. The fax phone number for this Group is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionists whose telephone number is (703) 308-0196.

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